HCN4 immunostaining in human (Alomone labs antibody)

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An abbreviated version of this protocol was published in Science Translational Medicine in Nov 2020

Ectopic HCN4 expression drives mTOR-dependent epilepsy in mice

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Detailed protocol

Preparation of human fixed brain sections:
Frozen (unfixed) OCT-embedded tissue were cut on cryostat (30 µm thick) and mounted onto glass slides.

Procedure:

1. Fix sections in **4% PFA for 5 min**, followed by 3x rinses in PBS.
2. Permeabilize in **PBS+ 0.3%Tx** for **20 min**.
3. Block **(10% goat serum, 0.3% BSA, 0.3% Tx in PBS)** for **2 hours** at RT.
4. Incubate in **1°antibodies** (diluted in **5% goat serum, 0.3% BSA, 0.1% Tx in PBS**) **o/n** at RT (in humidity chamber).
5. Wash in PBS+0.1%Tx for **3x10 min**.
6. Incubate in **2°antibodies** for **2 hours** at RT.
7. Incubate in DAPI for 5 min.
8. Wash in PBS+0.1%Tx for **3x30 min**.
9. Rinse in PBS, airdry, and coverslip with mounting media.

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Bordey, A. and Nguyen, L. (2021). HCN4 immunostaining in human (Alomone labs antibody). Bio-protocol Preprint. bio-protocol.org/prep840.
2. Hsieh, L. S., Wen, J. H., Nguyen, L. H., Zhang, L., Getz, S. A., Torres-Reveron, J., Wang, Y., Spencer, D. D. and Bordey, A.(2020). Ectopic HCN4 expression drives mTOR-dependent epilepsy in mice . Science Translational Medicine 12(570). DOI: 10.1126/scitranslmed.abc1492

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